EVIDENCE OF A PROTOPLASMIC NETWORK IN THE OENOCYTES OF THE SILKWORM.

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In the study of cell morphology the need of discovering some structural framework within the protoplasm has long occupied the mind of the investigators of this field. This subject has received consideration in books published by the late Mr. H. M. Bernard and Dr. Emil Rohde. Independently they have come to the same conclusion, namely, that all tissues and their constituent cells are permeated by a structural network. There is considerable evidence of such a network. In the nucleus the tangle of linin filaments have long been observed. Intracellular filaments have been abundantly demonstrated by a host of workers. Bernard and Rohde have brought out the very significant fact that syncytial tissues are permeated by such networks. The structural character of the cytoplasm has long been a field of controversy. Of the three different early theories—the Fibrillo Reticular Theory, Butschli's Foam Theory, and Altmann's Granular Theory—none has received universal acceptance. The discovery of mitochondria by Benda, Meves, Duesberg and others in all types of cells, has led to considerable speculation as to whether these elements can be truly associated with the linin filaments and their chromatin granules. Also whether they too have any architectural significance. These questions have been largely answered by the work of Michaelis, Chambers and the Lewises by staining the mitochondria of live tissue with Janus Green, and also the cell dissection work of Chambers and Kite. Chambers in his summary, draws the conclusion that the mitochondrial threads have tensile and elastic properties; however, this conclusion is qualified by the statement that these networks are not stable elements but that they reconstruct themselves by chemical or mechanical forces in response to changed conditions. In this paper an attempt will be made to show that in one particular case this network has a definite honeycomb structure and that the mitochondria and the linin filaments are continuous.

The silkworm œnocytes proved to be the most available material. These cells are large ductless glands found in the body cavity of all immature insects. The arrangement of these glands is in metamerical groups on both sides of the abdomen in conjunction with the spiracles. The cells are usually separate spherical individuals which lie enmeshed or suspended in a tangle of fine tracheæ with which, however, there is no closer connection than that of contact.

The origin of these cells is ectodermal. They arise in the form of a chain by amitotic division from one cell ventro-caudad of each spiracle. From this chain they migrate to their final positions. Before the egg hatches the ultimate number of cells in each group is reached and no further division takes place. These glands persist often into the imaginal stage. The secreting of the cell is a periodic function that takes place even in the embryo. While in the process of secreting the nucleus forms a honeycombed structure and from this long mitochondrial filaments extend to the periphery of the cell. The papers of Holland and Stendell contain good œnocyte reviews and nearly complete bibliographies.

The study of the mitochondria from an architectural point of view has largely been confined to the investigation of the origin of the highly specialized nerve and muscle fibrils. The œnocytes afford an opportunity of studying these structures comparatively free from the specializing influences of mechanical stresses or pressures. The cells originate while the egg is still in a semiplastic state. Immediately on being formed they float away in the body fluid till they come to rest in the delicate meshes of the trachea. For this reason any elemental structure in the cells of the primitive ectoderm might persist through the intervening generations into the make-up of the œnocytes.

With the exception of the eggs the œnocytes are the largest cells to be found in the insect body. This of course has the obvious advantage of making them easy to study. On the other hand when one considers that they are many times the size of the parent ectodermal cells, the question naturally arises as to whether a primitive intracellular network would meet this demand for expansion by the addition of more network or by the stretching of the original. From the observations of the œnocytes it would appear that the latter process is the one that occurs. The network is very open and it is this feature of size that makes it so very distinct.

The direct or amitotic division of these cells brings out certain other interesting conditions. In the writer's preparations the nuclear membrane appears to be totally absent. The very fine granular or colloidal structure of the cell, when brought out by high powers and the dark field illuminator show the nucleus and cytoplasm to have exactly the same physical make-up. The line between the two elements of the cell is shown only by staining. This would indicate that the nucleus and the cytoplasm differ in their chemical make-up rather than in structure as is the case in cells dividing mitotically.

The drawings accompanying this paper will show more clearly than any description the honeycomb structure of the nucleus. Reconstruction of the nucleus in clay makes it even more distinct. The process of formation can be traced in the sections. When the cell is not secreting the nucleus is spherical. It consists at this period of a mass of chromatin granules with which are interspersed about an equal number of drops of the secretion. The drops at this stage are little larger than the chromatin granules. The secreting process begins by the throwing out from the nucleus of several pseudopodia-like processes. These extend in a zigzag way in every direction. The chromatin and the secretion disperse along these lines till at length no large bodies of nuclear material remain. The nucleus has then the appearance of the aforementioned honeycomb. The edges are distinct lines and the vertices coincide in alignment. Around the outer edges of the structure there extend continuations of the edges of the honeycomb. These continuations are usually fine straight or sharply angular lines of particles. They have a striking resemblance in character to the spireme structures of the mitotic nucleus only they invariably follow straight or angular lines.

These long fine filaments respond to the test for mitochondria. They were first found in sectioned material stained with Mayer's alcoholic carmine. This material had been killed and fixed with heat and hardened in alcohol. Some of the same material stained in Hansen's Iron Hæmatoxylin gave clearer results. Benda's modification of the liquid of Flemming as as given by Eklof gave good results with Hansen's Iron Hæmatoxylin and Benda's Alizarin Crystal Violet.

The vital staining of the live cells with Janus Green did not prove to be a very satisfactory method. The cells showed the honeycombed nucleus very clearly but the color reaction for the mitochondrial granules was destroyed by the yellow tint of the cytoplasm. However, the mitochondria could be made out as masses of blue-green granules and filaments. The best strength for the Janus Green (Grubler) proved to be one part in one hundred thousand used in equal amounts with the body fluid.

A feature of these mitochondrial filaments is that they are often grouped around a central vertex. The vertex is analogous to and usually in alignment with the vertices of the honeycomb. It was found possible to measure the angles around these points when both arms lay in the same optical plane. The angular measurements fell into two distinct groups: (1) Angles approaching sixty degrees as a maximum, and (2) a lesser group of angles with an average measure of one hundred and ten degrees. Between four and five hundred actual measurements were taken from about twenty-five slides, from as many different individual larvæ.

Let us consider these data speculatively. From these two angles there can be constructed a hypothetical framework that is ideal from a structural point of view. The unit of such a structure is a tetrahedron. All the angles in one plane in an equilateral tetrahedron are of sixty degrees. The only other angle found in a structure of such units is of one hundred and eight degrees. Any attempt to conjure up a generalized or ideal structural basis of protoplasm is a piece of pure fancy. Yet the angles of the œnocytes fall in with such a plan and the honeycomb structure of the nucleus follows exactly the same lines.

It must be born in mind that the existence of any architectural basis of protoplasm is still a mooted question. This paper is purely speculative to the extent that it is based on the hypothesis that such a structure does exist. The object in taking this for granted is to show by the apparent figures in the œnocytes what would be the simplest character of such a structure. That these peculiar geometrical figures exist is plain to be seen. Whether they have a structural function is open to question. Their geometrical formation might be taken as indirect evidence of such a function. One of the remarkable points is the continuous character of the linin filaments and the mitochondria. This point of the continuous character of the linin is one of the postulates of the protomitomic theory as expounded by Mr. Bernard.

The work here recorded was done and the paper prepared in the Entomological Laboratory of Stanford University by me as holder of the Bernard Scholarship for 1914–1915 in Insect Histology.

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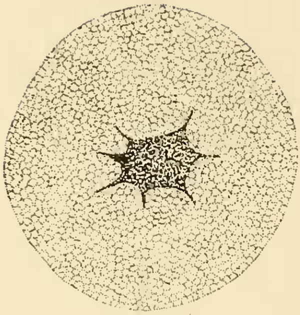
EXPLANATION OF PLATE XXIII.

Fig. 1. Oenocyte in which the process of secreting has just commenced.

Fig. 2. Oenocyte in which the nuclear material is moving out along definite lines.

Fig. 3. Section of an Oenocyte in which the honey-comb structure is appearing and in which only small masses of nuclear material remain.

Figs. 4, 5 and 6. Oenocytes in which sections of the honey-combed nuclei are shown. Note the fine terminal filaments which are mitochondria.



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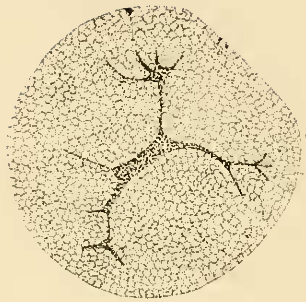

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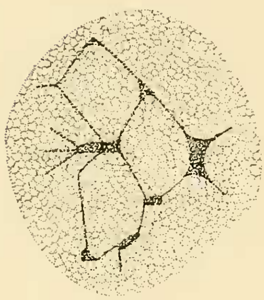

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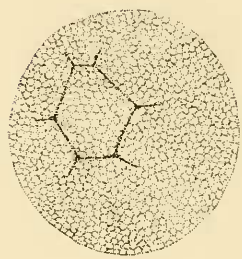

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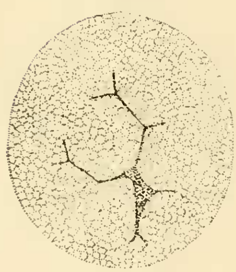

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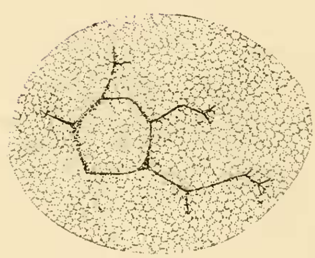

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